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Biodiversity of Calliphoridae Flies in South Rajasthan in Relation to Their Importance in Forensic Entomology

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Abstract: The analysis of the insect community present on a decomposing corpse can often provide valuable forensic insights, especially the estimation of the time of death, or postmortem interval (PMI). Life cycle of forensic insect act as clocks which starts within few minutes or hours after death. Udaipur has a semi-arid and vegetative climate. Forensically important flies directly affected from particular environmental condition. The present results highlight the particularities of local insect fauna corpses from Southern Rajasthan and their possible role in forensic entomology. The main objective of this study was to collect necrophagous insects colonizing the corpses as well as to gather information about their potential role in crime investigation and know the succession and decomposition pattern of forensically important fauna of Calliphoridae on carcass. The different life stages of Calliphoridae were observed during experiment and also compare with another forensically important families of Diptera like Sarcophagidae, Mucidae etc. Calliphoridae is most abundant fly that visit the carrion.

Keywords: Forensic Entomology, Postmortem interval, Carcasses, Calliphoridae

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Introduction

Forensic entomology is the science of collecting and analyzing insect evidence to aid in forensic investigations. The main application of forensic insect is in the determination of the minimum time since death in cases of suspicious death, either by estimating the age of the oldest necrophagous insects that developed on the corpse, or by analysing the insect species composition on the corpse. Forensic entomology

has many dipteran insects that infect the dead human material, these insects visiting on dead bodies and lay their egg on dead remains, especially Calliphoridae and Sarcophagidae family of flies visit the carrion firstly. Calliphoridae fly visit the carrion for food material and lay eggs on corpse. By using the development chronology of this fly we can estimate postmortem interval and can estimate time of death.

When any death is occurred in ecosystem then firstly Blowflies visit the corpse, followed by other insects. Calliphoridae flies initiate their life cycle on corpse so knowing which insects are present, and which life stage they are in, can determine how long ago a death took place. Estimation of PMI requires information on insect's biology, phonology and habitat preferences. The main objective of insect succession studies is to examine the colonization patterns of insects in a particular area, often with the goal of obtaining data that can be applied to forensic investigations involving insects in the event that a death occurs in a similar environment (Kreitlow, 2009). In order to precise estimation of PMI, indicator species from Diptera and Coleopteran are collected from corpse for the estimation of PMI. Insects colonize cadavers in a foreseeable sequence, called insect succession. Another application of forensic entomology in recognizing if body has been moved from crime scene to another place (Reed, 1958). Forensic entomologists use insects and other arthropods to clarify legal issues. There are several reasons for using Diptera insects in death investigations: insects visit carrion at first, the arthropod fauna on a corpse changes in a successional sequence as modifications of the cadaver occur (decomposition stages) and the fauna on a corpse is often ignored when investigators examine the death scene (Catts and Goff, 1992). It is also observed that the arthropods feeding on carrion form a distinct faunal succession associated with the various stages of decay of carcass (Smith, 1986). Knowledge of the habits, developmental rates, taxonomy and morphology, geographic distribution of each species involved can give useful information in determining the PMI (Catts and Goff, 1992). When other methods are not capable to provide appropriate information about time of death then life cycle of insects plays a massive role in estimation of Postmortem interval, Postmortem interval is based on the development time of forensically important dipterans and on the chronological succession pattern on the corpse during decaying process. In many countries of oriental region like India and Malaysia found

Parasarchophaga ruficornis (Kumara *et al.*, 2012).

Pathologists can estimate the time of death based on many biological parameters: rigor mortis, lividity, changes in the chemical constituents of body, postmortem cooling, autolysis of tissue, and decomposition due to bacterial activity in the body. However, these parameters are not sufficient beyond about 72 hours after death then only forensic entomology is useful tool to detect the criminal activities with the help of life cycle and succession pattern of forensic insects (Mohammed *et al.*, 2015). Forensic entomology is the application and study of insect and other arthropod biology to crime matters. It also involves the application of the study of arthropods, including insects, arachnids, centipedes, millipedes and crustaceans to legal cases. Forensic insects are primarily associated with death investigations; however, it may also be used to detect drugs and poisons in dead body, determine the location of an incident, and find the presence and time of the infliction of wounds. Insect evidence may show that the body has been moved to a second site after death, or the body has been distributed at some time either by animals, or killer returning to the crime scene.

The main objective of this work was to study the calliphoridae flies biodiversity in particular area with stages of decomposition as well as to identify the calliphoridae visiting on the carrion. To generate a baseline data using carrion as a source of decomposition, studies on calliphoridae colonization was carried out at Southern Rajasthan. Present study will provide an idea of occurrence of forensically important Calliphoridae in studied area, and succession pattern of forensically important Calliphoridae fly on carrion and such study will provide strong platform to solve the criminal investigations with the help of insects.

Materials and Methods

Study Site:

The present study was conducted in Udaipur district of South Rajasthan (lies in between 24°58"

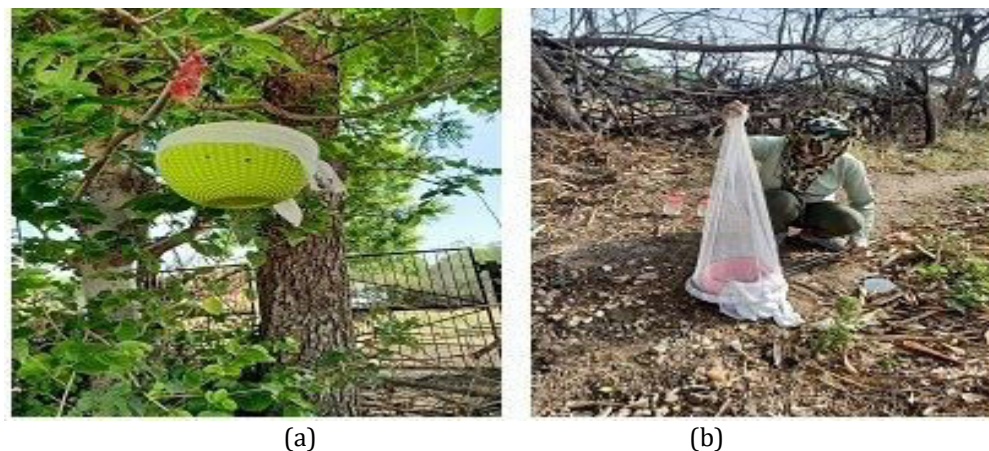


Fig. 1: (a) setup of fly traps suspended at 2-3 meters height to capture adult flies (b) collection of flies from field using swiping net.

N and 73° 68" E; 598 meter above sea level) to observe the succession pattern of life stages of Calliphoridae fly on the carcass. A setup of cage having goat liver and rat carcasses was made at different places of Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan (India).

Experimental Design:

To capture the forensic calliphoridae flies from the studied area as a sample Goat Liver (purchased from a butcher shop) and dead mice were used. The sample was put in the 2 plastic cages having many holes and was kept in a bushy area of the college (Fig. 1a). Observation was taken daily around 8:00 am for 12 consecutive days.

Collection Sampling & Identification:

Observation on visiting insects was done once a day. Adult flies were captured by swiping net (Fig. 1b) and preserved in 70% ethanol for further identification. Egg and larvae were collected from carcasses by brush and transferred to different cages for further observation of life cycle of Calliphoridae. Collection was done until skeletization of carcasses. Photography of captured calliphoridae flies were done with the help of Lieca Stereo zoom microscope for further identification and identified by using taxonomic key (Lutz *et al.*, 2017).

Environmental Conditions:

During the study average of maximum and

Minimum temperature and humidity were recorded daily by portable hygrometer--Maximum and minimum temperature (31.0 °C, 12.0 °C) and humidity (89%, 25%) (Fig. 2).

Succession and Decomposition Pattern:

Various decomposition stages and arrival insects data were compiled from captured insects to observe the succession pattern and distribution on their arrival preference.

Results and Discussion

The study showed that Calliphoridae (blow fly) visit at first and the maximum number of flies visiting on carrion was also Calliphoridae. Total fifty-six Calliphoridae flies specimens (*Chrysomya megacephala*, *Chrysomya albiceps*, *Lucilia papuensis*, *Cynomya mortuorum*) were collected from carcasses (Fig. 3) Calliphoridae species were the predominant ones. The present study showed that at first *Chrysomya megacephala* (Calliphoridae) fly visited at carrion.

Succession and Decomposition pattern:

Throughout this study, four Calliphoridae species were identified in association with the decomposition of the goat liver meat carcasses. The succession and decomposition pattern of collected Calliphoridae flies were observed from goat liver (Tables 1, 2). Early colonizers arriving during the fresh stage of decomposition was *Chrysomya megacephala* followed by *Chrysomya*

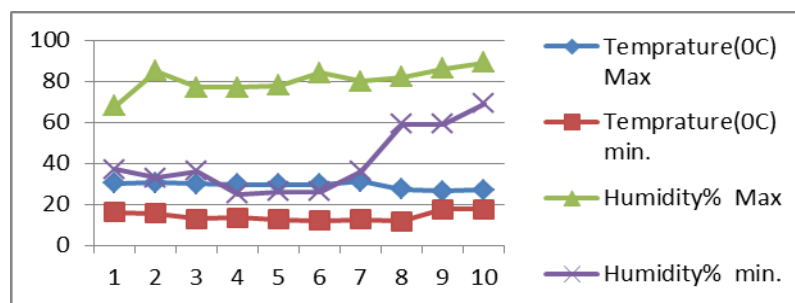
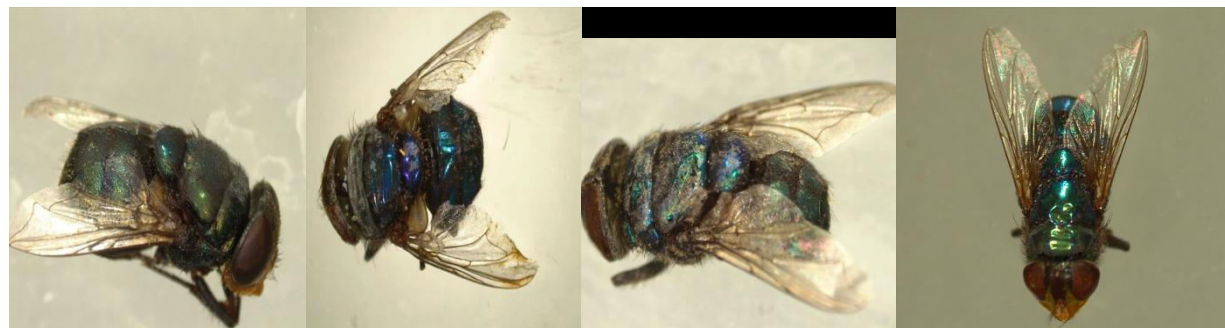


Fig. 2: Temperature and relative humidity.



Chrysomya albiceps

Cynomya mortuorum

Lucilia papuensis

Chrysomya megacephala

Fig. 3: Collected Calliphoridae fauna from sample.

albiceps, *Lucilia papuensis* and *Cynomya mortuorum* (Diptera: Calliphoridae) (Figs. 4, 5).

Fresh Stage (0–1 d):

The fresh stage commenced directly after the carcass was kept, and it was associated with soft torsos and no foul odor. There was a decrease in the body temperature from 29.4°C to 20.4°C. *Chrysomya megacephala* (Diptera: Calliphoridae) was the first colonizer in fresh stage.

Bloated Stage (2–4 d):

At this stage, the body color of carcasses changed from white and became darkened, a foul odor was being emitted from the meat carcasses. *Chrysomya megacephala*, *Chrysomya albiceps* and *Cynomya mortuorum* were species observed and recorded during the bloated stage. There was an increase in the total number of Calliphoridae flies on carcasses.

Active Stage (5–7 d):

There was a slight decrease in the total number of Calliphoridae flies (*Chrysomya albiceps*, *Lucilia*

papuensis, *Cynomya mortuorum*) during this stage.

Advanced Stage (8–9 d):

This stage was characterized by drying out of the skin and loss of the softness of meat carcasses. *Chrysomya albiceps*, *Lucilia papuensis* and *Cynomya mortuorum* were observed during this stage.

Dry Stage (10–12 d):

There was a decrease in the total number of calliphoridae species recorded. *Chrysomya albiceps*, *Lucilia papuensis* and *Cynomya mortuorum* were observed during this stage.

The present study showed that Calliphoridae flies are first colonizer on carrion in Udaipur region of south Rajasthan. The maximum number of visitors is also Calliphoridae followed by Musca, and Sarcophagidae. Calliphoridae and Sarcophagidae flies play important role as forensic investigator. However, the results are opposed to the results given by Singh *et al.* (2016), who have

Table 1: Succession of calliphoridae flies on goad liver carcasses

Date	Day	Calliphoridae	Temperature (°C)		Humidity%	
			Max	min.	Max	min.
20/10/2019	1	Adult <i>(Chrysomya megacephala)</i>	30.2	16.2	68.0	37.0
21/10/2019	2	Adult <i>(Chrysomya megacephala)</i>	30.6	15.6	85.0	33.0
22/10/2019	3	Adult Maggot <i>Chrysomya megacephala, Chrysomya albiceps, Cynomya mortuorum</i>	30.0	13.0	77.0	36.0
23/10/2019	4	Adult <i>Chrysomya megacephala, Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	29.6	13.5	77.0	25.0
24/10/2019	5	Adult <i>Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	29.6	12.6	78.0	26.0
25/10/2019	6	Adult <i>Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	29.5	12.0	84.0	26.0
26/10/2019	7	Adult <i>Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	31.0	12.5	80.0	36.0
27/10/2019	8	Adult <i>Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	27.2	11.6	82.0	59.0
28/10/2019	9	Adult <i>Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	26.5	17.5	86.0	59.0
29/10/2019	10	Adult <i>Chrysomya albiceps, Lucilia papuensis, Cynomya mortuorum</i>	27.1	17.5	89.0	69.0

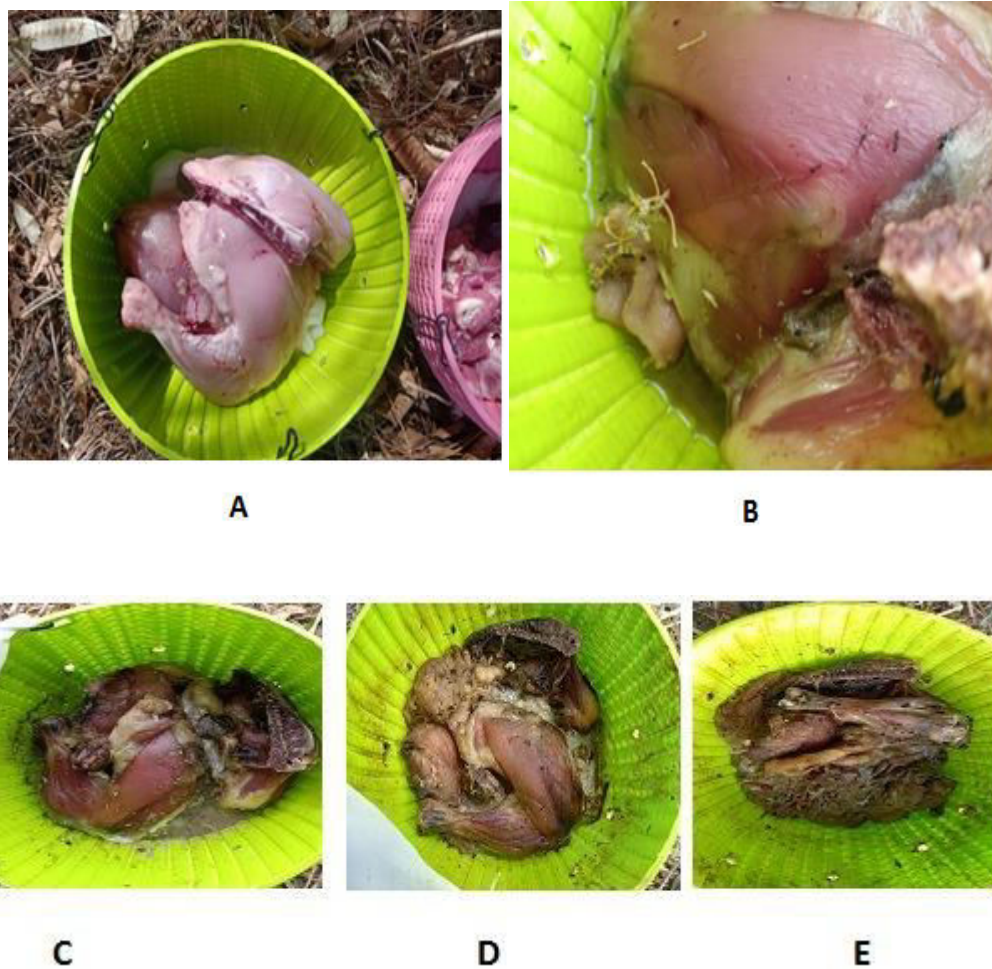


Fig. 4: Different stages of decomposition of meat carcass (A) fresh stage (B) blotted stage (C) active decay stage (D) advanced decay (E) dry stage.

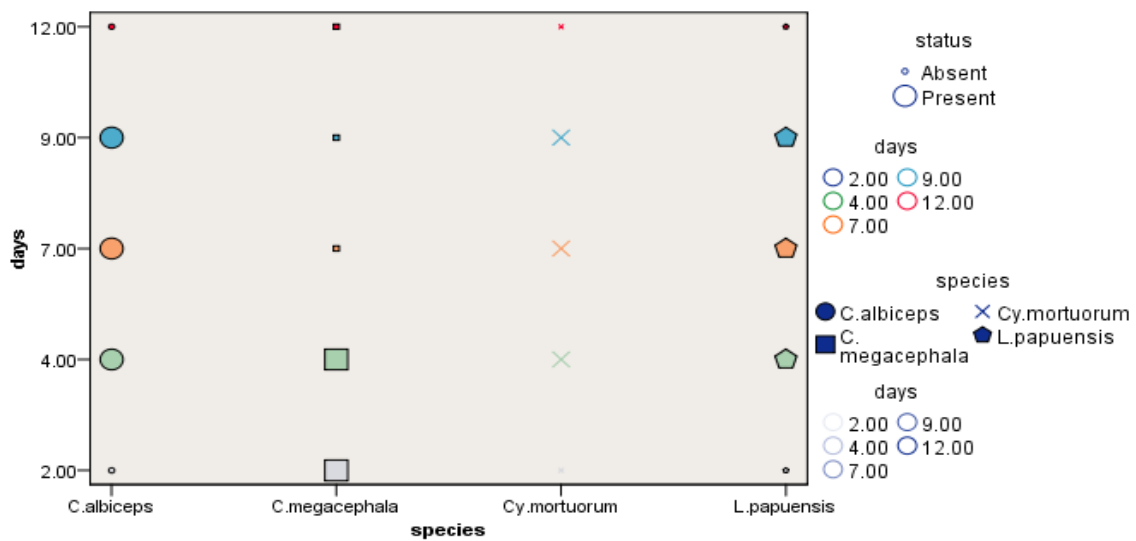


Fig. 5: Occurrence of Calliphoridae species during days of decomposition.

Table 2: Stage of decomposition and presence of Dipteran species

Stage of Decomposition	Period (Days)	Time of sampling	Presence of dipteran species
Fresh	01	8.00-9.00 AM	Yes
Bloated	2-4	8.00-9.00 AM	Yes
Active	5-7	8.00-9.00 AM	Yes
Advance	8-9	8.00-9.00 AM	Yes
Dry	10-12	8.00-9.00 AM	Yes

reported the Ecological Succession pattern of Diptera on the carcass of Laboratory bred rats and found that Sarcophagidae family is the first colonizer on carcass. Ali *et al.* (2013) studied forensically important Diptera associated with dog carcass in Pakistan, and reported that *C. rufifacies* (Calliphoridae) is first visiting member on carrion.

Arnaldos *et al.* (2001) observed the Succession pattern of forensically important insects from the chicken carcasses and recorded Calliphoridae (69.4%), Sarcophagidae (7.3%), Muscidae (16.9%) and Fannidae (6.4%) in the southeastern Iberian peninsula. Campobasso *et al.* (2001) observed five decomposition stages in pig carcasses during the experiments-- fresh, bloated, decay, decay-adipocere and dry. Changes in body temperature and appearance were depended on climatic factors. The observed succession pattern showed similarities with other studies since two principal groups were represented (Diptera and Coleoptera) (Tullis and Goff, 1987; Anderson, 2000). Biological Decay of carcasses is usually affected by environmental factors. In addition to biological mechanisms, the decomposition of a Carcass is determined by the chemical, physical and climatic variables of its geographic location (Merritt *et al.*, 2009).

In the present study Calliphoridae, *Chrysomya megacephala* is the first colonizer and the maximum number of flies on carrion. Sarcophagidae fly is the second colonizer followed by Muscidae but Muscidae is more in number as compared to Sarcophagidae.

Criminal activities solved by forensic entomo-fauna:

1. A study at a residential area of Ludhiana, Punjab India on March 30, 2015, a 40-year male dead body was found with maggots and pupae of *Chrysomya megacephala* on the body (Anika *et al.*, 2018).
2. A study at a residential area of Ludhiana, Punjab (India) on 22nd April 2015, a 26-year-old male body was found with different instars of larvae of *Chrysomya rufifacies* and beetles belonging to the family Dermestidae and Cleridae i.e. *Dermestes maculatus* and *Necrobium rufipes* (Anika *et al.*, 2018).
3. A study at a residential area of Ludhiana, Punjab India on 6th July 2015, a 32-year-old unknown male body was found with Dipteran flies *Chrysomya megacephala*, *Chrysomya rufifacies*, *Phormia regina* and maggots of *Chrysomya albiceps*. An unidentified wasp was also collected from dead body (Anika *et al.*, 2018).
4. A study at a residential area of Punjab India on 5th July 2015, a 50-year-old male body was found with larvae of *Chrysomya albiceps* on all over the body (Anika *et al.*, 2018).

Conclusion

The present study revealed that Calliphoridae flies reach on carrion in a few hours and lay eggs on carrion. The present study also showed that Calliphoridae flies members are the first colonizer and maximum in number that visit on carrion. Insect succession patterns from animal and human

carrion can provide useful information to determine the time of death (PMI). The data from our study could be very useful for further forensic investigations in India. Such studies are needed to develop a larger geographical database of insect succession on carrion in a variety of habitats and in all regions in which forensic entomology is used. This study gives baseline data regarding insect colonization during various stages of decomposition which helps in the identification of insects associated with goat and rat corpses recovered in that particular area if forensic entomology is utilized to solve the crime. So, from this experiment, it is concluded that Calliphoridae *Chrysomya megacephala*, *Chrysomya albiceps*, *Lucilia papuensis* and *Cynomya mortuorum* species were the predominant species in this area.

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